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~~The views, opinions~~ and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

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13. ABSTRACT (Maximum 200 words) : In the present research period we have developed a procedure for coupling PCR-mutagenesis to natural transformati that genetic analysis of structure-function relationships can be extended to genes from any organism as long as they provide a phenotype that is readily characteri We have extended analysis of *Acinetobacter* genes to *hca* genes which encode proteins that convert hydroxycinnamates to protocatechuate. The hydroxcinnama are important environmental chemicals because they are produced by plants in response to stress. Among the chemicals degraded by *hca* gene products is chlorogenate, a compound that resists digestion in animal feed stocks. In addition to the *hca* structural genes, the *Acinetobacter* chromosome contains *hcaK* whi encodes an apparent transporter and *hcaR* which is a transcriptional repressor. HcaR a member of the important MarR family of transcriptional regulators that gc drug resistance in bacteria.

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(1) List of papers submitted or published under ARO sponsorship **during this reporting period**. List the papers, including journal references, in the following categories:

(b) Papers published in peer-reviewed journals

1. **David M. Young, Ruben G. Kok, and L. Nicholas Ornston.** 2002. Phenotypic Expression of Polymerase Chain Reaction-Generated Random Mutations in a Foreign Gene After its Introduction into an *Acinetobacter* chromosome by natural transformation. Methods in Molecular Biology vol 182: In Vitro Mutagenesis Protocols, 2nd ed. J. Braman, ed.

2. **Smith, M. A., V. B. Weaver, D. M. Young and L. N. Ornston.** 2003. Genes for chlorogenate and hydroxycinnamate (*hca*) are linked to functionally related genes in the *dca-pca-qui-pob-hca* chromosomal cluster of *Acinetobacter* sp. strain ADP1. Applied and Environmental Microbiology 69:524-542.

2) "Scientific personnel" supported by this project and honors/awards/degrees received

Donna Parke, David Young (Ph.D. received, December 2001), Michael Smith, Nicholas Ornston

3.) Report of inventions. None

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(4) "Scientific progress and accomplishments" (Description should include significant theoretical or experimental advances)

Acinetobacter sp. strain ADP1 is a nutritionally versatile bacterium that exhibits extraordinary competence for natural transformation. This genetic trait greatly facilitates genetic analysis and in the past has been coupled to random PCR-mutagenesis in order to identify how amino acid residues influence function in enzymes and transcriptional activators associated with catabolic pathways. We now have extended this capability to genes from other organisms, in this case Pseudomonas putida, by creating a docking site that allows PCR-amplified P. putida DNA to be integrated into the Acinetobacter chromosome under control of a promoter causing constitutive expression of genes contained in the integrated DNA. Phenotypic expression of the P. putida DNA allows identification of mutants in which the function of the P. putida gene has been altered. This development opens many opportunities for determination of how structure influences function in proteins from a full range of organisms.

Our research interest in Acinetobacter has focused upon its ability to metabolize diverse compounds that serve as nutrients for it in the natural environment. The fundamental understanding that emerges from this work is important because modification of such metabolic systems will be important in developing procedures for bioremediation. In addition, modification of the Acinetobacter pathways affords opportunities for synthesis of important chemicals by biological means.

Central to many catabolic pathways is the reaction catalyzed by protocatechuate 3,4-dioxygenase, and our past research has centered upon pcaHG, the structural genes for this enzyme. Many spontaneous mutants lacking pcaHG contain large deletions extending through flanking regions of DNA, and characterization DNA corresponding to the deletions has revealed a cluster of about 50 genes associated with dissimilation of compounds produced by plants. Among these are straight chain dicarboxylic acids and hydroxycinnamic acids which are building blocks in suberin, a protective polmer produced by plants in response to stress.

During the present research period we have focused attention on a portion of the gene cluster containing hca genes which encode proteins essential for conversion of hydroxycinnamic acids to protocatechuate. Among the enzymes involved is an unusual hydratase/lyase which is of particular interest because it converts abundant natural products such as ferulate to value added products such as the aldehyde vanillin. Among the hca genes is hcaG which encodes an esterase

that cleaves chlorogenate to quinate and caffeate (caffeate is another hydroxycinnamate). Both of quinate and chlorogenate are metabolized through protocatechuate. Chlorogenate esterase is of considerable significance because its substrate presents a major problem in animal feedstocks because it is not metabolized by animals.

Downstream from the hca structural genes we discovered a gene cluster designated hcaKR. HcaK is a transporter similar in sequence to four other transporters we have discovered in our genetic analysis of Acinetobacter. HcaR is a transcriptional regulator which, unlike previous regulatory genes we have characterized, is a repressor. This is immediately significant because mutations in HcaR cause constitutive expression of enzymes that provide useful products from hydroxycinnamates. Furthermore, HcaR is a member of the MarR family of transcriptional regulators of drug resistance. We have developed physiological procedures for selection of MarR mutants, and we anticipate that analysis of such strains will provide general information about how this family of proteins exerts control over transcription.

5. Technology transfer. None.